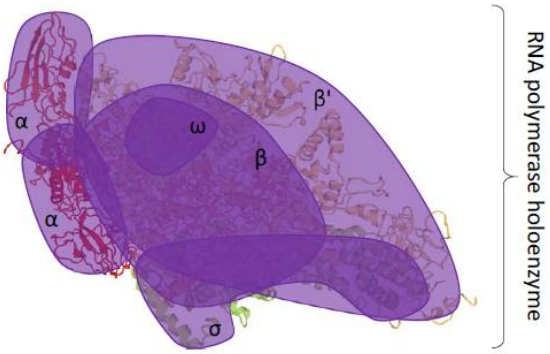
Transcription

Ribonucleic Acid

- Polynucleotide
- Ribose sugar vs deoxyribose (no OH at 2'C)
- Bases: A, C, G, U (methylated thymine)
- Single-stranded
- Forms: mRNA, tRNA, rRNA, others

RNA Polymerase



- Core RNA polymerase: 5 subunits
  - 1 × β – catalyses polymerisation
  - 1 × β' – helps bind DNA
  - 2 × α – interacts with other proteins
  - 1 × ω – function unknown
- σ subunit – finds 'promoters'
- Active site: β + β'
- Holoenzyme: All units (core + σ)

Transcription Process

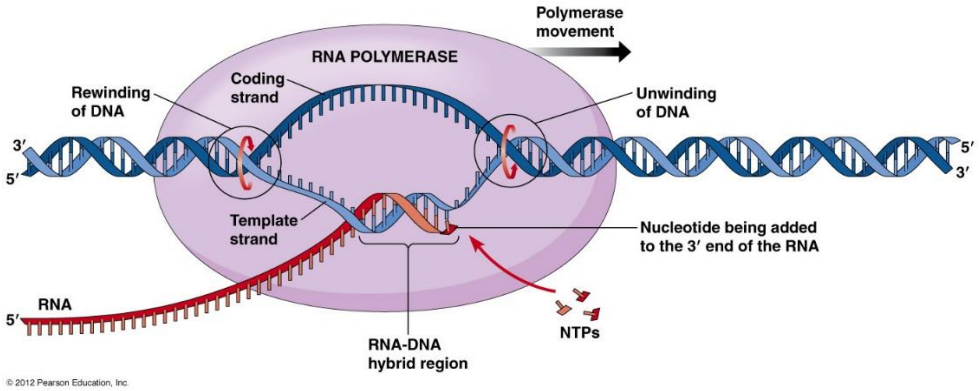
Initiation

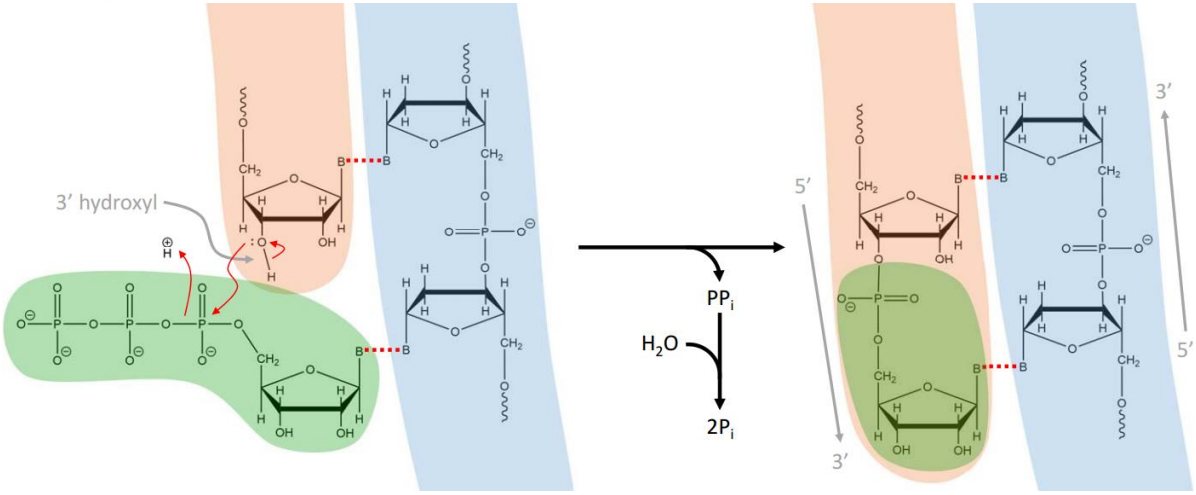
- Coding/sense strand: Contains promotor sequences; transcribed
- Template/anti-sense strand: Complementary sequence made from this template; copied

- Promoters: Sequences in DNA that tells RNA polymerase where to start transcription
  - Consensus sequences (i.e. common bases in promoters): TTGACA (approx. -35 region), TATAAT (approx. -10 region)
  - Found on coding strand (sense strand) – before +1 start site
    - Downstream more +ve
    - Upstream more -ve e.g.
    - Upstream → downstream = 5' to 3' on coding strand
    - E.g. +1 is more downstream than -10

1. σ subunit locates promoter sequences on coding strand
2. Template strand (anti-sense strand) read from 3’ to 5’ → complementary strand made 5’ to 3’ (exactly like coding strand)
3. Transcription bubble: DNA melted and strands split downstream of promoter (towards +1 and more +ve) to allow RNA polymerase to read
    - Caused by σ subunit at start

Elongation

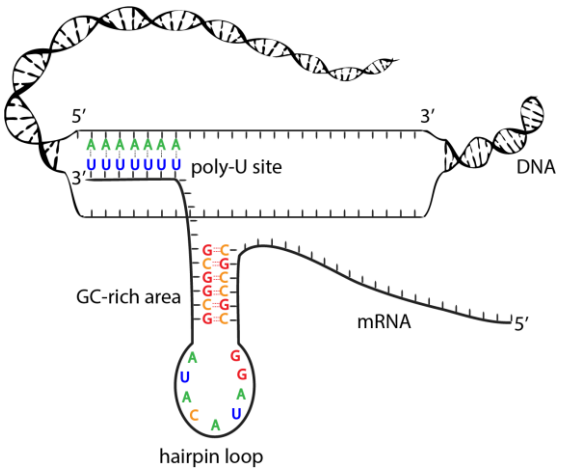




- Formation of phosphodiester bonds w/ NTP joined at 3’ OH
    - Hydrolysis of NTP → NMP + PPi → 2Pi + energy!
- Promoter clearance: After ~10 nucleotides joined, σ subunit falls off and RNA polymerase core moves along DNA
    - NusA protein binds to RNA polymerase (involved in elongation and termination)

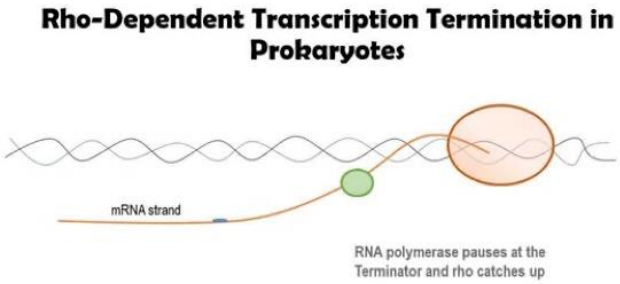
Termination

- Factor independent: GC-rich region at 3’ end



- Does not rely on proteins or other factors
- When transcribed onto mRNA, causes hairpin loop within mRNA via H-bonding of bases (3 H bonds per base) → slows mRNA → removal of mRNA from DNA by breaking weak A-U bonds at poly-U site of mRNA

- Factor dependent: Rho (p) factor



- Rho attaches to sequences on mRNA and hydrolyses ATP to move
- GC region inside termination sequence slows down RNA polymerase
- Rho catches up w/ RNA polymerase, interacts w/ NusA and winds mRNA around itself → destabilises mRNA and DNA bonding

Controlling Gene Expression

Factors Affecting Expression

Not affected by:

- No. of copies of genes: only 1 copy of each gene in prokaryotes
- Rate of translation of mRNA
- Rate of core enzyme for RNA
- Shine Dalgarno (translation) closer to consensus

Affected by: Things that alter frequency of transcription → transcriptome and proteome

- Strength of promoter i.e. TTGACA, TATAAT consensus sequences
- Repressor and activator binding sites
  - E.g. lac operon

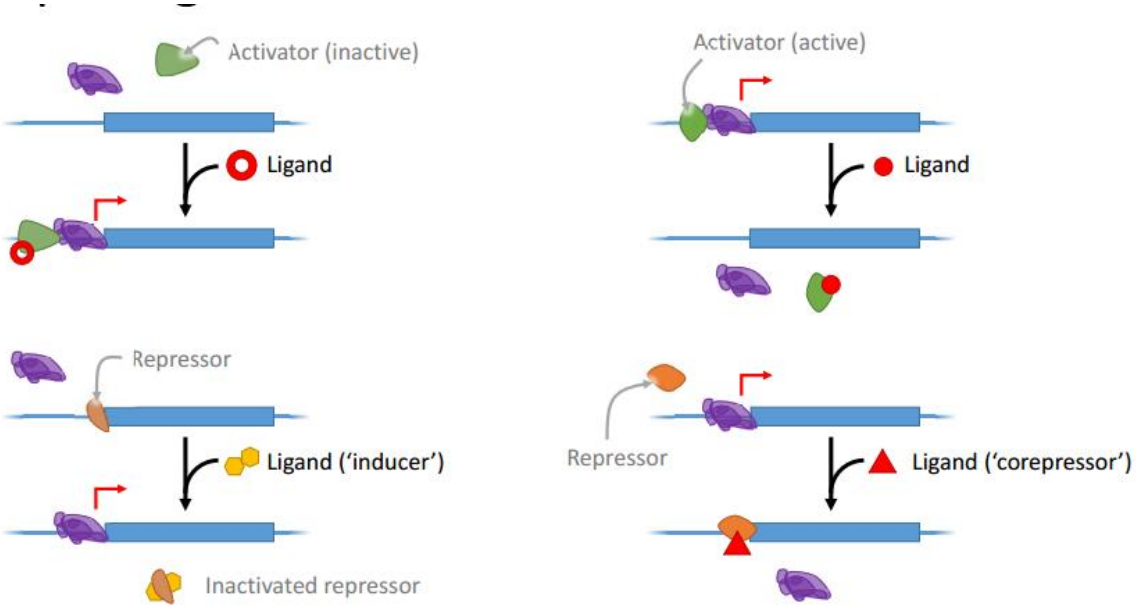
Types of Genes

- Constitutive gene: Gene expressed all the time, at constant level

    - High constitutive expression: High expression all the time
        - Close to σ^{70} consensus → more often expression
        - E.g. glucose transport
    - Low constitutive expression/'housekeeping': Low expression all the time
        - Not as close to σ^{70}
- Regulated expression: Expressed at particular time
    - Specific consensus sequence for specific σ (not σ^{70})
    - E.g. heat shock chaperone
    - Proteins disable σ^{70} to allow other σ factors to transcribe
- Inducible gene: Gene expression varies in levels depending on situation

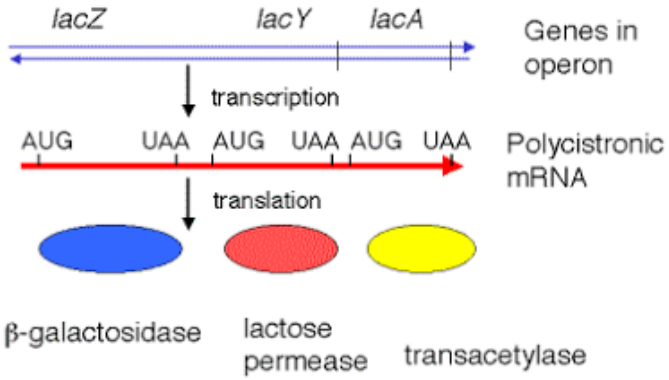
Repressors, Activators, Ligands

- Negatively regulated gene: Repressor prevents transcription
- Positively regulated gene: Absence of activator prevents transcription
- Repressors and activators change confirmation due to ligand binding → may be able/not able to bind to DNA → affect transcription (start/stop)



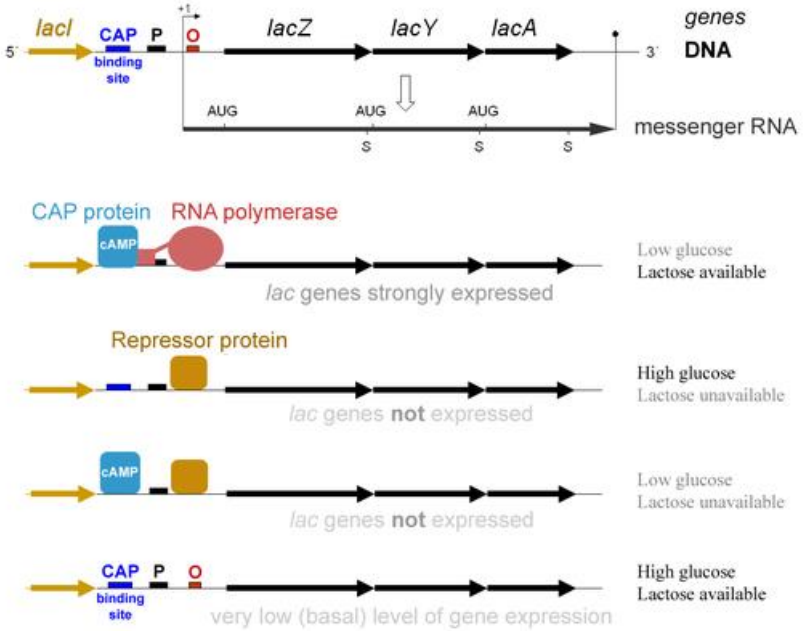
Lac Operon

- Operon: Cluster of genes controlled by 1 promoter
    - Not all products are translated however; separate start and stop codons for each gene in mRNA after transcription



- Metabolises lactose when there is little glucose
- Consists of lacA (unknown), lacY (lactose transporter), lacZ (b-galactosidase)
- LacI gene constitutively i.e. always expressed → lac repressor protein
    - Binds to operators downstream of promoter → blocks initiation of transcription through steric hindrance (even though RNA pol binds it jumps off)
        - Very little (but some) transcript made → lactose permease and b-galactosidase
- When [lactose] is high:
    - Lactose → allolactose via b-galactosidase
    - Repressor binds to allolactose → change conformation → frees up operators
- When [glucose] is low:
    - [Cyclic AMP/cAMP] increases
    - Cyclic AMP binds to cyclic AMP regulatory protein (CRP)/catabolite activator protein (CAP)
    - cAMP-CAP binds upstream of promoter region (doesn't block)
    - Contacts α subunit of RNA polymerase at promoter
    - → Activator → Weak promoter converted to stronger one

The *lac* Operon and its Control Elements



[Lactose]	[Glucose]	Level of Transcription
High	High	Free lac operon promoter but WEAK promotor → low level of transcription
High	Low	Free lac operon promoter and activator binded → high level of transcription
Low	High	No transcription as operator blocked
Low	High	No transcription as operator blocked

Trp Operon

- Codes enzymes that make tryptophan
- Normally trp repressor is inactive → free operator → transcription
- Low [trp] → inactive repressor
- High [trp] → trp binds to repressor → binds to operator → no transcription